CYTOLOGY AND SYSTEMATIC POSITION OF PORPHYRIDIUM CRUENTUM NAEGELI

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Porphyridium cruentum, named by Nägeli in 1849, has had a systematic history equaled by few plants. It had previously been called at various times Thelepora, Tremella, Sarcoderma, and Byssus. Agardh (cit. Brand) named it *Palmella cruenta*, and under this name Hassall classified it with the Palmellaceae. Indeed, Nägeli himself placed it in this group, and there it was kept by Kützing in his Tabulae Phycologicae (1849–71) under the name given it by Agardh. Rabenhorst seems to have been the first to place it in the Porphyraceae (1868), and he was followed in this four years later by Wood. Cooke in 1884 returned it to the Palmellaceae. Wolle likewise placed Porphyridium in the Chlorophyceae and found it to be identical with *Protococcus miniatus*. On the other hand, Schmitz (cit. Brand) considered it related to the Florideae, and Gaidukov (cit. Engler and Prantl) put it in the Bangiaceae. In 1902 Chodat returned it to the Chlorophyceae and would place it near Schizogonium. West two years later believed that Porphyridium belongs in the Myxophyceae and is allied to Aphanocapsa, while Hansgirg (cit. DeToni) made the genus but a species of Aphanocapsa and called it *A. cruenta*. Oltmanns in 1905 put Porphyridium once more in the Chlorophyceae. He was not certain as to its exact position but placed it supplementary to the Scenedesmaceae. DeToni classified Porphyridium as one of the Myxophyceae belonging to the family Glaucophyceae. Brand in 1908, as a result of his work on this plant, believed that it belongs to the Bangiaceae, and in this he is generally followed by the systematists, Engler and Prantl, West, who changes his original position, and Collins. Tilden, however, keeps Porphyridium in the Myxophyceae. While Kufferath got some results very different from Brand's, he agreed with the latter as to its systematic position, although he suggested that in the contingency of its having no chlorophyll it be placed with the red bacteria. Brand cited Borzi as being in favor of putting Porphyridium with Protococcus, Richter as favoring putting it with Trentepohlia, while Klebs would have it as a questionable member of the Pleurococcaceae.

The descriptions of Porphyridium differ almost as much as its various systematic positions. Nägeli, working unfortunately with dried material, described it as follows:

"Cells flattened, in surface view round or somewhat polygonal from lateral pressure, with a lateral thin confluent sheath, united in one-layered

free-lying families; divisions in varying vertical planes; all generations fully developed and alike; cell contents purple.

"Type *P. cruentum* (*Palmella cruenta* Ag.), the only known species.

"The blood-red gelatinous layer consists of larger or smaller one-layered plates, whose cells seen from the surface appear rounded and mostly somewhat angular. The thickness of the cells is in dry specimens one third to one fifth the breadth. The thin sheaths run together in a structureless jelly in which the cells are imbedded. The sheaths are one third to one fifth, more seldom up to one half, of the lumen. The true wall is very thin.

"The cell content is colored by erythrophyll. It looks beautifully purple and agrees in color with *Porphyra vulgaris*. I could not see a nucleus in it."

On procuring some living material he amended his description somewhat:

"Cells spherical or polyhedric with tolerably thin confluent sheaths, united in a somewhat gelatinous layer; divisions varying in all directions of space or exceptionally only in vertical planes. . . . This genus is distinguished from Palmella by the erythrophyll in the cell content." He added in a note: "Further I saw in the fresh plant, often in every cell, a whitish granule (a chromatophore filling itself with starch), such as the other Palmellaceae possess."

In 1875 Mer found starch in Porphyridium, and in the same year Saint-Léon found no trace of sexual reproduction and only simple multiplication through the division of the vegetative cells. Schnetzler (1878) reported that the red coloring matter disappears when the alga is pickled in a borax solution, leaving the color green, and Nebelung (1878) that the red pigment has a spectrum which may be considered as a modified spectrum of the pigment of Phormidium. Schmitz added considerably to our knowledge of this alga by describing in it a star-shaped chromatophore, which, like those of the Bangiaceae, Bacillariaceae, and Rhodophyceae, contains no starch; and also a colorless centrally located pyrenoid and an eccentric nucleus. Later he reported that "the special cell membrane is repeatedly formed anew on the single cells, the old membrane is torn through on one side and stripped off as a stalk, at first sharply delineated and later becoming more and more formless gelatin." On the other hand, Oltmanns described the cells as being imbedded in formless jelly.

Brand reported that the chromatophore is not typically star-shaped but often in wet weather is round, and that the star-shape, when it does occur, comes from its being indented with the peripherally located granules and vacuoles. These granules he took to be cyanophycin granules, though he records that they are not stainable with acid carmin, which is generally held to be the most typical stain for such granules. The coloring matter, he found, is floridean red and varies only in its intensity. He was unable to find any green modification. The pyrenoid is described as being ring-shaped

and often hard to see, and in "house cultures" it even disappears in most cells. In regard to the nucleus he said: "Although now the existence of a nucleus is *a priori* very probable, I could, after completely dissolving the sheath, never with certainty show one. The nucleus-like structures which one sees in living as well as in fixed stained material, are not only in regard to size, form, and position very variable, but appear sometimes single and sometimes many. All usual methods of staining have given me, through repeated investigations, very uncertain results."

Kufferath, utilizing the technic of bacteriology, was able to get a pure culture of Porphyridium to grow in various gelatinous media. The alga growing thus showed a great increase in size, at times reaching a diameter of 24 μ, and showed somewhat of a variation in its method of division. Two daughter cells sometimes developed within the body of the mother cell, and even tetrads occurred. Kufferath denied the existence of a pyrenoid in Porphyridium and stated that what has been taken for a pyrenoid is an optical effect due to a convergence of the light rays by the plastid. In regard to the nucleus also his findings are quite different from Brand's. He writes: "The nucleus, which has been seen only by Schmitz, is colored by the usual stains; it is oval, somewhat refractive and applied against the cell wall; it is small and we have not been able to distinguish its intimate structure."

A most obvious explanation for this divergence in the results of the various investigators would be furnished if the case of Porphyridium were analogous to that of Protosiphon and Botrydium. Different species of plants, no matter how much alike externally, would hardly give identical results on an intimate investigation, especially if their ancestry were diverse and they had evolved along parallel lines. While it is possible that more than one genus has been investigated under the name of Porphyridium, and this possibility should not be overlooked in future investigation of this much studied but little known alga, the facts at present do not substantiate this hypothesis. The present investigation has often shown in the same plant two characters, each of which has been described and had its existence denied by some of the aforementioned authors, whose views were just the opposite in regard to its accompanying character.

The diameter of Porphyridium in the material studied varies from 5 μ to 9 μ, the smaller cells almost uniformly being in the resting condition. The jelly secreted by each cell forms an individual sheath about that cell and, when division takes place, the two daughter cells are in the same sheath, which follows the constriction of the cells quite intimately, and lengthens as the cells draw apart. The portion of the sheath between the two cells becomes drawn out into a strand or stalk (figs. 10, 11, 39). As these cells were originally within the sheath of the mother cell, which itself was on a stalk, we frequently find the mother stalk branching into two

daughter stalks. However, no case was found of more than two cells being borne on branches on a single stalk, which would indicate that the stalk does not persist through three generations. Indeed, if growth is inhibited, the stalks tend to blend into a common gelatinous sheath and appear as in figure 12. The stalks are elastic. It is quite a common instance for two sister cells to have stalks of different lengths, and in each instance observed the longer stalk was the thinner, as if it had been stretched out. Brand observed that the pressure of the cover glass would flatten out the jelly, which would resume its original shape if the presure were removed.

Löffler's flagellum stain will show these stalks very well, a little better as a rule if pyrogallic acid be used in place of tannic acid. A good method of proceeding is to place a small amount of rapidly growing alga on a slide and allow it to dry until it has lost all of its water content. It should then be covered with the mordant and heated for ten minutes over a water bath.

Much clearer results, however, have been obtained by allowing the alga to dry as described above and then fixing in the following solution:

Sat. sol. anhydrous ferric bromide in ether ..... 1 part
Molar sol. pyrogallic acid in ether ..... 2 parts

The water in the gelatin will cause the solutes to ionize, and hence ink will be precipitated within the gelatin. This makes a good mordant for gentian violet and safranin. If the jelly has dried too much it can be impregnated with ink by having the fixing agent washed off with water. Another good fixing and staining agent for jelly is:

Sat. sol. gentian violet in 95% alcohol ..... 1 part
Formalin (40% formaldehyde) ..... 1 part

This stains the jelly a dark red or purple and leaves the cell contents colorless.

The chromatophore is typically star-shaped in the resting cell (figs. 12, 13, 18). However, in the cells that are rapidly growing, the enlargement of the cell does not seem to be followed by an equal increase in the size of the chromatophore, so large vacuoles appear at its periphery. Its shape can then be best described as amoeboid.

The chromatophore is of a dark red color, almost that of clotting blood. If, however, the plant is allowed to stand for a short while under water, the red coloring matter can be seen dissolved in the water and the gelatinous mass becomes grass-green.

The centrally located body, which has almost uniformly been called a pyrenoid whenever it was observed, and will be considered such in this paper, is colorless in the living cell and appears only as a light spot in the chromatophore. Unstained it could very readily be mistaken for an artefact due to the refraction of light by the chromatophore. However, the "convergence of light rays" of Kufferath takes Heidenhain's haematoxylin very well and is not indifferent to gentian violet and safranin (figs. 2, 9, 18,

27). The pyrenoid is generally spheroidal in shape, though when the cell starts to divide it lengthens and becomes somewhat angular. As a rule it stains uniformly dark, though at times it appears ring-shaped with a relatively unstained center (figs. 25–27).

A single eccentrically located globule, a trifle smaller than the pyrenoid, has been frequently noted in Porphyridium. It can be seen very easily in the living specimen and has been observed to fragment as the water content of the cell increases, the fragments arranging themselves about the chromatophore. Except in its reaction toward acid carmin it seems to act as if it were cyanophycin. In general, we find, it takes the usual nuclear stains, haematoxylin, gentian violet, and safranin.

Picric acid has, on the whole, given the best results as a fixing fluid. If it is washed out in running water, the chromatophore will be dissolved and the pyrenoid and "nucleus" left without being obscured by any other cell structure. Ten minutes in the acid is enough for the fixation, and from fifteen minutes to twelve hours will do for the washing out of the fixative. Mordanting for one hour in iron alum and staining for a like period in haematoxylin have given the best results with this stain. Staining for ten minutes over a water bath is sufficient for anilin-gentian violet and anilin-safranin, and the specimen can then be decolorized by allowing it to stand over night in methyl alcohol. Another very successful fixative and mordant is:

Pyrogallic acid (25% aqueous sol.)	10 parts
Ferrous sulphate (sat. sol.)	5 parts
Fuchsin (sat. alc. sol.)	1 part

Van Ermengen's osmic acid process has given only fair results. Whenever the material was fixed in either of Flemming's fluids, or when fixed in picric acid and hardened in alcohol, the chromatophore stained so densely that it was impossible to distinguish anything in the cells clearly. Flemming's triple stain has given very fair results.

The chromatin, consisting of a single eccentric granule surrounded by a clear space in the cell (fig. 18), is typical of the resting stage, a stage described by Brand as "wasserarm." The cell, however, if dried, is useless as far as any clear results are concerned. As the cell prepares for division, this granule enlarges and begins to fragment, assuming the various shapes shown in figures 18–27. No hard and fast rule can be laid down for establishing a sequence of forms in this breaking up, as there are many forms which do not fit well into any series that could be arranged out of the others, although some of the shapes occur in many cells. The "U" shape is perhaps the most common (figs. 21, 22), and it is not at all unusual to find the fragments united in a line (figs. 23, 24) or in a ring (fig. 20). Frequently the pieces in drawing apart leave trails which have a striking resemblance to the mitotic spindle (fig. 25), which resemblance seems to be purely accidental. As an end result of this fragmentation the chromatin is distributed in the

form of small elongated granules about the periphery of the chromatophore (figs. 27, 28). These granules then fuse end to end and a well tangled spireme results (fig. 29). A striking thing about this spireme is the way various strands lie parallel to each other. One is greatly tempted to see in this a conjugation or perhaps a splitting of the spireme. However, the members of a pair do not necessarily go to different cells. The spireme breaks into pieces of varying lengths, and these segments frequently withdraw into two distinct masses before the cell has started to constrict. More often, however, the chromatin is constricted in two with the cell (figs. 30, 31), and it is nothing unusual to see strands extending some distance into each daughter cell when the cells are connected only by a narrow isthmus (fig. 32). Two granules of chromatin occur regularly at the poles of the dividing cell at the maximum distance from the plane of constriction (figs. 30, 31, 32). These granules occupy these definite positions too often for this arrangement to be due to a mere fortuitous placing of waste chromatin, though what function is served is not at all clear. In the majority of cases the segments of the spireme in newly divided cells lie alongside the new wall formed by the constriction (fig. 33). If any spindle fibers were present in this division, the technic used caused them to be dissolved, as no traces of them were found. The evidence at present indicates that a typical resting stage is not necessary between successive cell divisions especially if the conditions are just right for rapid growth.

The dividing cells studied came from an agar culture in Chodat-Grintzesco solution. For a culture to thrive it must not be in a liquid medium or kept in the dark. No organic energy-yielding compound was necessary for rapid growth.

In searching for mitosis in a primitive or degenerate plant, the investigator is exposed to the danger which beset the late centrosome hunters, of mistaking a chance resemblance for a homologue. The eccentrically placed globule seems certainly to be chromatin, and whether we call it a nucleus or a nucleolus depends upon the relative flexibility with which we use these terms. In regard to its fragmentation it resembles the nucleolus, though if it is the nucleolus it contains all of the chromatin at this stage, which is not typical. The amount of chromatin apparently increases greatly as the fragmentation progresses, and this increase is too great to be explained by the increase in the precipitation of the stain on the increased surface exposed. Some of the stainable material may come from the fragmented pyrenoid. The chromatin is arranged in a typical spireme, which breaks up into segments of diverse sizes which may safely be considered as analogues of chromosomes. There is no lining up on an equatorial plane or any indication of the segments splitting and having their halves drawn to opposite poles. This method of nuclear division may be recorded as mitotic, but the mitosis is quite primitive and of an exceptional kind.

The bearing of this method of nuclear division upon the systematic posi-

tion of Porphyridium must be uncertain until more is known of the nuclear history of the Bangiaceae. The resting stage is certainly unlike anything known in the Myxophyceae, though the later stages show a certain resemblance to this group. The whole process is a bit too primitive for the Chlorophyceae. In regard to its other characteristics Porphyridium resembles the Bangiaceae, and it would be best to keep it in this group.

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Summary

1. Divergent results of the various investigators are probably due to their working with plants at different stages of growth rather than to their working on plants of different species.

2. The jelly is homogeneous only after a prolonged period of inactivity of the plant. In growing material the cells are born on gelatinous stalks.

3. The chromatophore is star-shaped only in the resting stages. Its red coloring matter can be extracted, leaving it green.

4. Porphyridium has a distinct, easily stainable, centrally located pyrenoid, which is generally spheroidal though sometimes ring-shaped.

5. In the resting stage Porphyridium has a single eccentric globule of chromatin homologous to a nucleus or nucleolus. Nuclear division is crudely mitotic.

6. Porphyridium had best be kept in the Bangiaceae at present.

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EXPLANATION OF PLATES

PLATE XX

These photographs were taken with a Gordon's photomicro camera. In figure 1, a Zeiss 2 mm. water-immersion objective and a Bausch and Lomb no. 10 eye-piece were used; in figures 2–12, a Zeiss 1.5 mm. oil-immersion objective and a Zeiss compensating ocular no. 6. The tube length was 160 mm. Magnification, × 400.

FIG. 1. Living cells showing cell division.

FIG. 2. Fixed in picric acid and stained in Heidenhain's haematoxylin.

FIG. 3. Same as figure 2.

FIG. 4. Fixed over a water bath with picric acid and stained with anilin-gentian violet.

FIG. 5. Fixed and mordanted with pyrogallic acid-ferrous sulphate, stained with anilin-gentian violet.

FIG. 6. Fixed as in figure 5. Not stained.

FIG. 7. Fixed as in figure 5. Stained with anilin-safranin.

FIG. 8. Stained by Van Ermengen's osmic acid-silver nitrate process.

FIG. 9. Fixed with tannic acid, stained with anilin-gentian violet.

FIG. 10. Fixed with a solution of pyrogallic acid and ferric bromide in ether, stained with safranin.

FIG. 11. Same as figure 10.

FIG. 12. Fixed in 1 part formalin and 1 part saturated solution of gentian violet in 95% alcohol, counterstained in Heidenhain's haematoxylin.

PLATE XXI

FIGS. 13–17. Living material showing increase in size and cell division. × 1070.

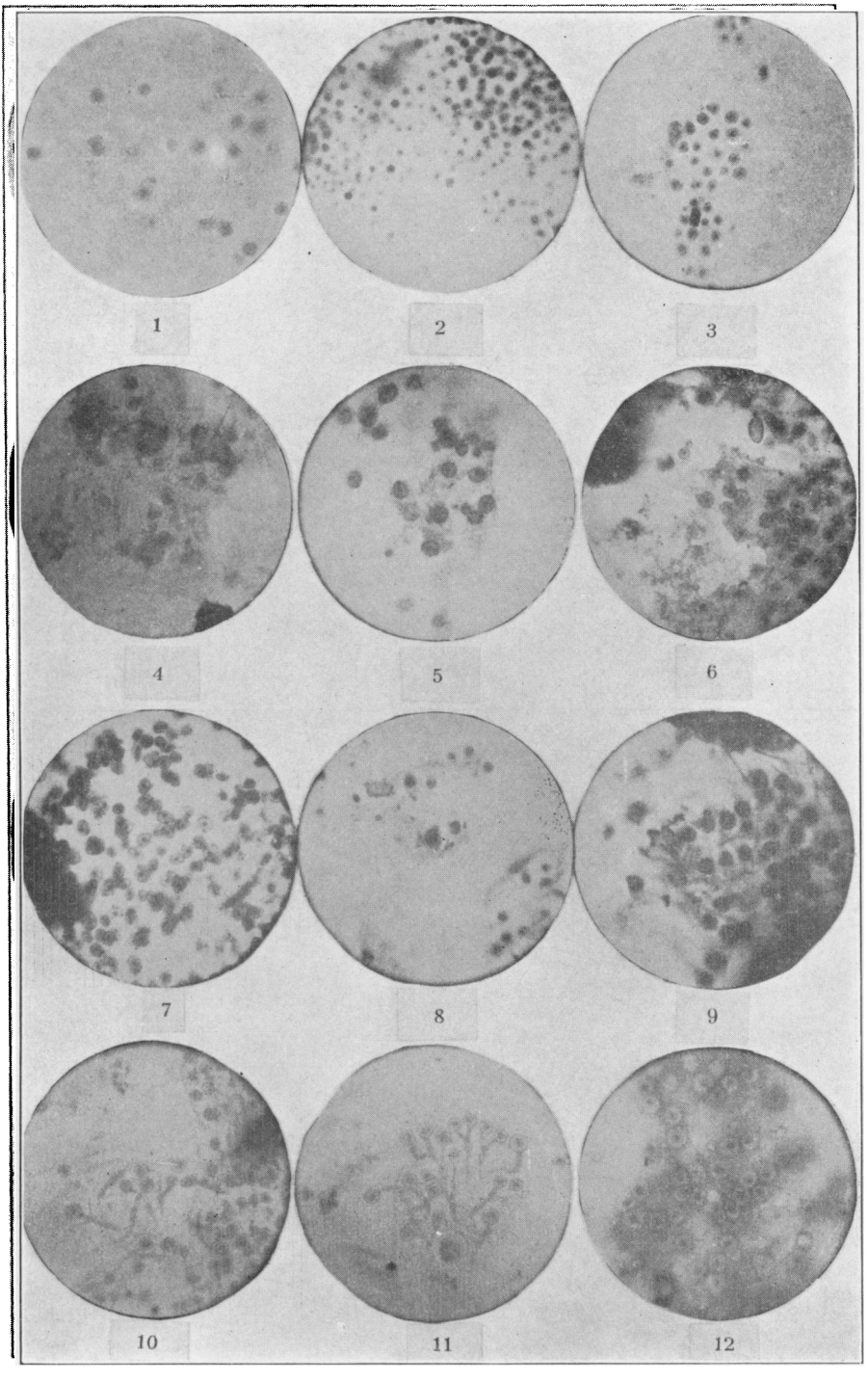
FIG. 18. Resting stage. × 1600.

FIGS. 19–27. Stages showing fragmentation of chromatin and changes in pyrenoid. × 1600.

FIGS. 28–34. Stages showing nuclear and cell division. × 1600.

FIGS. 35–38. Stages showing reassembling of chromatin. × 1600.

FIG. 39. Cells connected by stalks. × 1600.



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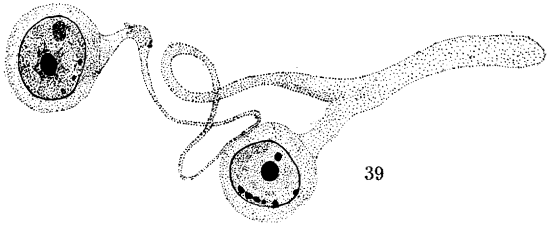

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